Opening of Self-Assembled Protein Nanocapsules Triggered by Low pH

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INTRODUCTION

Vaults are nanoscale, ribonucleoprotein capsules (41 nm × 72.5 nm [1]) comprised primarily of 96 self-assembled copies of one 104 kDa protein, termed MVP (major vault protein). When deposited on poly-lysine-coated mica and imaged using cryoelectron microscopy, vaults appear to "open" into flower-like structures with eight rectangular 'petals' [2]. Upon closer examination, each 'flower' consists of a central ring with hooks that attach each petal to the center. The flowers are usually seen in pairs, suggesting that each whole vault is composed of two flowers, folded so that the ends of their petals touch. It is likely that vaults in cells open and close in response to cellular signals, reversibly encapsulating and releasing their contents. However, whether vaults open into flower-like structures *in vivo* is unknown.

The biological function for vault nanocapsules, which are ubiquitous intracellular components of eukaryotes, is unknown; yet they may prove useful for drug delivery and for compartmentalized materials synthesis. Whole vaults self-assemble from MVP subunits which have been cloned and over-expressed [3], thereby providing an attractive system for the study of biological self-assembly and a potentially versatile platform for biomaterials design In the present study, our aim is to design mechanisms for reversible vault assembly/disassembly in order to control the encapsulation and release of materials; the first step in the process is to identify conditions which promote vault opening.

EXPERIMENTAL

Fluorescence Spectroscopy

Experiments discussed here have been designed to investigate solution conditions which cause vault opening. Initially, the intrinsic fluorescence intensity of tryptophan present in the vaults was utilized to investigate the effect of pH on vault conformation. In order to change the pH environment, vaults in 20 mM MES at pH 6.5 were diluted at least 15-fold into 85 mM Tris (pH 6.5- 9.0) or citrate phosphate buffer (pH 2.6-6.5, total concentration 55-85 mM), and incubated at room temperature for 1 hour. Free tryptophan was treated similarly as a control. Samples were excited at 295 nm and the emission intensity was recorded at the peak wavelength.

Solution conditions believed to trigger vault opening were further examined using fluorescence quenching with acrylamide. Samples were pH-treated as described above. Then increasing aliquots of a 2M acrylamide stock were added to the solution and allowed to equilibrate for 10 minutes before being scanned. The data obtained in these studies were fit to a quenching model for multiple tryptophan-containing proteins [4] using a non-linear least squares fit.

Quartz Crystal Microbalance

The use of the quartz crystal microbalance (QCM) is based on the converse piezoelectric theory whereby applying an oscillating electric potential across the non-conducting quartz crystal surface propagates an acoustic wave within the surface which has a certain resonant frequency. After adsorbing

a monolayer of vaults onto the surface, we can change the pH of the running buffer and simultaneously monitor the resulting changes in mass (given by a change in frequency of the quartz crystal oscillation) and in protein height off the surface (given by a change in resistance to the oscillation due to viscous losses.) Since it can independently measure protein mass and compactness on the surface, in this way, the QCM provides us with an additional tool for monitoring the effect of pH changes on absorbed vault conformation.

RESULTS AND DISCUSSION

Fluorescence Studies

By monitoring the intrinsic fluorescence of tryptophan residues of vault proteins as a function of pH, evidence was gathered that suggested a partially open vault conformation at low pH. At pH 3.4, the buffer-subtracted emission intensity of vaults, corrected for the pH dependence of free tryptophan fluorescence, was found to increase by (60±2)% relative to the corrected fluorescence intensity of vaults at pH 6.5, indicating a more open vault conformation.

Further examination of the effect of pH 3.4 on vault conformation was carried out with fluorescence quenching studies of vaults versus free tryptophan using acrylamide. Since the quenching constant (K_Q from our model [4]) describes the frequency of the encounter between acrylamide and an excited tryptophan molecule, it is therefore a kinetic measure of the exposure of the tryptophan residues [5]; the higher the value of K_Q, the faster and easier the quenching. From these studies, the quenching constant for whole vaults at pH 6.5 was found to be 5.8±0.3% of that for free tryptophan at the same pH. However, the quenching constant for vaults at pH 3.4 was found to be 12.8±0.7% of that for free tryptophan at this low pH, which is 2.2±0.3 times greater than quenching of whole vaults at pH 6.5. The enhanced quenching exhibited at low pH indicates a conformational change of the vaults has taken place to allow the quencher easier access to the tryptophan residues originally embedded in the vault shell.

QCM Surface Studies

Surface studies of adsorbed vaults have been carried out to further elucidate the nature of vault conformation change at reduced pH. By monitoring the changes in QCM frequency and resistance, evidence was obtained that vaults adsorb onto self-assembled monolayers (SAMs) in an approximate monolayer. Subsequent changes in pH indicate that vaults dissociate along their equator, with half of the vault particle being released into solution and the remainder flattening on the surface. If the pH is again raised to 6.5, the adsorbed vault half assumes a more upright, cup-like conformation. The hypothesized scenario for the pH response of vaults on a surface is shown pictorially in Figure 1.

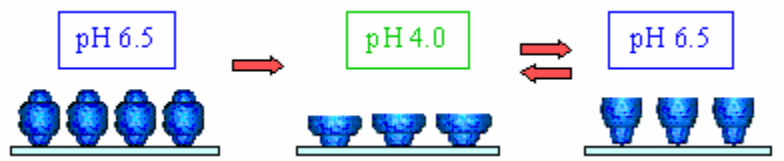

Figure 1: Pictorial representation of vault conformational on surfaces in response to low pH, based on interpretation of QCM results.

Negative-stain transmission electron microscopy (TEM) images of vaults exposed to low pH support our interpretation of these QCM results.

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